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Translating gastric cancer genomics into targeted therapies

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ABSTRACT

Gastric cancer is a common disease with limited treatment options and a poor prognosis. Many gastric cancers harbour potentially actionable targets, including over-expression and mutations in tyrosine kinase pathways. Agents have been developed against these targets with varying success- in particular, the use of trastuzumab in HER2-overexpressing gastric cancers has resulted in overall survival benefits. Gastric cancers also have high levels of somatic mutations, making them candidates for immunotherapy; early work in this field has been promising. Recent advances in whole genome and multi-platform sequencing have driven the development of molecular classification systems, which may in turn guide the selection of patients for targeted treatment. Moving forward, challenges will include the development of appropriate biomarkers to predict responses to targeted therapy, and the application of new molecular classifications into trial development and clinical practice.

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1. Introduction

Gastric cancer is the fifth most common cancer worldwide, and one of the leading causes of cancer-related mortality (International Agency for Research on Cancer, 2014). The treatment options for gastric cancer are limited, and patients invariably have a poor prognosis- patients with Stage 3 and 4 gastric cancer have 5 year overall survival rates of 9.2–19.8% and 4.0% respectively (National Cancer Institute, 2014). Despite recent breakthroughs in the use of targeted therapy in many other cancers, similar advances in gastric cancer have been slower. Part of the challenge arises from the heterogeneity of gastric cancers on a clinical, histologic and molecular level, which demands an individualized approach (Tan, 2015).

Historically, gastric cancer has been subdivided by histologic subtype via the World Health Organisation (WHO) or Lauren classifications, each with distinct clinical and epidemiologic features. The Lauren classification divides gastric cancers into intestinal and diffuse types, accounting for 54% and 32% respectively (Polkowski et al., 1999). Intestinal gastric cancers tend to be associated with

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environmental factors like Helicobacter pylori infection, occur in the antrum, and are often preceded by intestinal metaplasia. Diffuse gastric cancers on the other hand are more poorly differentiated, occur in younger patients, have a poorer prognosis and are found in inherited conditions. The WHO classification splits gastric cancers by their resemblance to metaplastic intestinal tissue (Dicken et al., 2005; Hu et al., 2012). More recently, the genomic study and characterisation of gastric tumours have given further insight into the pathogenesis of these cancers, and identified new potential therapeutic targets. This may pave the way for the development of personalised prognostication and treatment.

2. Molecular classification

The increasing efficacy and accessibility of sequencing has allowed multi-platform sequencing in large numbers, and in turn drives the development of classification systems based not on histopathology but on molecular features. The landmark Cancer Genome Atlas (TCGA) study performed sequencing of 295 gastric cancer samples on 6 different molecular platforms. Based on this, gastric cancer was clustered into 4 groups- Ebstein-Barr virus (EBV) positive (9%), tumours with microsatellite instability (MSI) (22%), genomically stable tumours (20%) and those with chromosomal instability (50%) (The Cancer Genome Atlas Research Network, 2014).

The EBV positive subtype, interestingly, showed a high level of non-silent *PIK3CA* mutations (80%), of which 68% were recurrent, as well as mutations in *ARID1A* (54%) and *BCOR* (23%). The rate of *PIK3CA* mutations in the other subtypes was 3–42%. There was also a high prevalence of DNA hypermethylation, particularly of the *CDKN2A* promoter, and amplification of the genes encoding immune checkpoint ligands PD-L1 and PD-L2. The MSI high subgroup, characterised by high levels of microsatellite instability without major chromosomal abnormalities, were enriched in tumours with hypermethylation, especially of the *MLH1* promoter, leading to *MLH1* silencing. The chromosomal instability subtype was associated with extensive somatic copy-number aberrations, and amplifications in genes involved in the RTK (receptor tyrosine kinase)-RAS pathway that lead to its activation. Finally, the genomically stable subtype, which lacked either chromosomal alterations or microsatellite instability, was high in *CDH1* and *RHOA* mutations.

These molecular analyses showed that each of the various subtypes had certain candidate therapeutic targets. The presence of *PIK3CA* mutations in EBV positive tumours suggest that these tumours may be particularly amenable to PI3K inhibitors. It was noted that the *PIK3CA* mutations that occurred in EBV positive gastric cancers were scattered over the gene, rather than concentrated over the kinase and helicase domains in exons 9 and 20, as was seen in the other subtypes. It remains to be seen whether these *PIK3CA* mutations truly have functional significance on the PI3K pathway. In addition, with new strides being made in immunotherapy, the prevalence of PD-L1 and PD-L2 over-expression in EBV positive tumours may also be of interest. In the genomically stable subtype, *RHOA* and *CLDN18* gene products are potential therapeutic targets, while in the chromosomal instability subtype, VEGF and other RTK amplifications highlight the possible role for RTK inhibitors like ramucirumab. MSI cases generally lacked targetable amplifications, although mutations in *PIK3CA, ERBB2, ERBB3, EGFR* and *ARID1A* were occasionally seen.

A similar undertaking by the Asian Cancer Research Group (ACRG) looked at 300 primary tumours, on which gene expression profiling, genome-wide copy number microarrays and targeted gene sequencing were done (Cristescu et al., 2015). In this study, gastric cancer was divided into 4 groups- MSS/EMT (microsatellite stable/epithelial-to-mesenchymal transition), which encompassed the outliers on the EMT distribution, MSI, MSS/TP53+, which had patients with intact TP53 activity, and MSS/TP53-, tumours which had functional loss of TP53. This population was unique in that long term follow up data was available. On a clinical level, these subtypes had prognostic value- with best prognosis in the MSI subtype, then MSS/TP53+, MSS/TP53- and then MSS/EMT. MSI tumours were more likely to be intestinal and diagnosed at an early stage, while MSS/EMT tumours were diffuse and more likely to recur. On a molecular level, the MSI subtype was confirmed to have hypermutation. Amplifications of *ERBB2*, *CCNE1* and *CCND1* tended toward mutual exclusivity in the MSS/TP53- subtype, which can be targeted by trastuzumab, CDK2 inhibitors and CDK4/6 inhibitors respectively. The MSS/TP53+ subtype showed a higher prevalence of mutations in *APC, ARID1A, KRAS, PIK3CA* and *SMAD 4*. In particular, sequencing revealed that of the 3 most common *PIK3CA* mutations, the ones in the MSI subtype tended to be H1047R mutations (A- > T), while in MSS tumours, E542 K and E545K mutations (G- > A) prevailed. These mutant *PIK3CA* proteins have been shown to cause oncogenic transformation in vitro, but it is not known yet which ones are more susceptible to PI3K inhibitors (Kang et al., 2005).

New genetic alterations and associations with particular tumour types continue to be identified, and contribute to our understanding of gastric cancer. For instance, *RHOA* hotspot mutations have been found to be common in diffuse (14.3%) but not intestinal-type tumours, with suggestions of a role as a driver of tumorigenesis. Other possible drivers that have recently been identified include *MUC6*, which codes for a mucoprotective mucin, *RNF43*, which negatively regulates WNT signalling, *CTNNA2*, involved in cell adhesion, and *GLI3* and *ZIC4*, both of which are involved in sonic hedgehog signalling (Wang et al., 2014). Recently, 5 recurrent fusion genes have been identified- one of them, *CLDN18-ARHGAP 26*, is seen in 3% of Asian Gastric cancers and is thought to contribute to the invasiveness of tumour cells (Yao et al., 2015).

These studies suggest that we should be moving towards molecular screening and classification of gastric cancers, to stratify them for treatment and prognostic purposes. Despite this, we are still some way from a consensus on the most relevant system.

3. Tyrosine kinase targets

Many of the promising molecular targets in gastric cancer are receptor tyrosine kinases (RTKs). Deng et al. profiled copy number alterations in gastric cancers and found that at least 37% of them harboured genomic alterations in RTKs that may be targets for agents that are currently available or under development. These included 9% of tumours with *FGFR2* alterations, 9% *KRAS*, 8% *EGFR*, 7% *HER2* and 4% *MET* (Deng et al., 2015).

3.1. HER2

HER2 is a transmembrane tyrosine kinase and a member of the epidermal growth factor receptor (EGFR) family, involved in the regulation of cell proliferation, adhesion, migration and differentiation. This occurs via heterodimerization with other members of the HER family, leading to activation of the RAS-MAPK and PI3K-AKT pathways. The *HER2* gene is located on chromosome 17q21 (Gravalos and Jimeno, 2008; Hudis, 2007). HER2 overexpression occurs in 15–30% of gastric cancers, and prevalence depends on the histology and location of the tumour- it is more common in the intestinal type (34% intestinal, 6% diffuse, 20% mixed) and in gastro-oesophageal junction tumours (32% in GEJ tumours vs 18% in gastric cancers) (Bang et al., 2010). HER2 positivity can be defined by protein expression on immunohistochemistry, and is obtained when there is strong membranous reactivity in >/ = 10% of cancer cells on surgical specimens or a cluster of five or more cells with

strong reactivity on a biopsy specimen. It can also be identified on fluorescence in-situ hybridisation (FISH), with a HER2:CEP17 ratio of >/ = 2 considered positive.

HER2 positive gastric cancers have been successfully targeted by the anti-HER2 monoclonal antibody trastuzumab, which works through inihibition of the MAPK and PI3K/Akt pathways to suppress cell growth and proliferation, as well as via the recruitment of immune effector cells responsible for antibody-dependent cellular cytotoxicity (ADCC). The Phase III ToGA study looked at the role of adding trastuzumab to chemotherapy in the first line of treatment in patients with HER2 positive advanced gastric cancer. Patients were randomised to fluorouracil based chemotherapy and cisplatin with or without trastuzumab. There was an overall survival benefit from the addition of trastuzumab- median overall survival (OS) 13.8 vs 11.1 months (HR 0.74, CI 0.60–0.91; p = 0.0046), with no increase in the rates of grade 3 or 4 adverse events (Bang et al., 2010). The objective response rate was also higher with trastuzumab, at 47% vs 35% in the standard arm. In the ToGA population, there was a proportion of patients (22%) who were FISH positive but IHC 0–1- these patients did not seem to benefit from trastuzumab. Based on the ACRG and TCGA classifications, HER2 amplification may be found more in the MSS/TP53- and in the chromosomal instability subgroups respectively.

Other anti-HER2 agents have been studied in the treatment of HER2 positive advanced gastric cancer. Lapatinib, a dual anti-EGFR and anti-HER2 tyrosine kinase inhibitor has been investigated. In the TRIO-013/LOGIC trial, patients with advanced or metastatic HER2 positive gastric cancer were randomised to capecitabine and oxaliplatin with lapatinib or placebo. The primary endpoint of overall survival benefit was not reached, with a median OS of 12.2 months in the lapatinib group and 10.5 months in the placebo group (HR 0.91, 95% CI 0.73–1.12, p = 0.35). Progression free survival (PFS) was also not significantly improved- median PFS 6.0 vs 5.4 months (HR 0.86, 95% CI 0.71–1.04, p = 0.10) (Hecht et al., 2013). In the second line setting, the Tytan trial compared paclitaxel alone with lapatinib plus paclitaxel, and again did not reveal significant benefits in median OS, PFS or time to progression (TTP) (Satoh et al., 2014). Recently, pertuzumab, a monoclonal antibody that binds the extracellular dimerization domain of HER2 preventing its dimerization, has shown benefits in the treatment of breast cancer. There is currently a Phase III trial, the JACOB trial, underway to at the efficacy of pertuzumab in combination with trastuzumab and chemotherapy (cisplatin plus a fluoropyrimidine) (NCT01774786). Another Phase III trial is looking at trastuzumab emtansine, an antibody-drug conjugate, compared to a taxane in patients with previously treated advanced HER2 positive gastric cancer (NCT01641939).

3.2. VEGF

Vascular endothelial growth factor (VEGF) overexpression is a common feature in gastric cancers, seen in up to 58% of cases (Oh et al., 2008). In the TCGA paper, recurrent amplification of the gene encoding ligand VEGF-A was particularly seen in chromosomal instability tumours, and in 7% of tumours overall. It promotes carcinogenesis by inducing angiogenesis and neovascularisation, and the VEFGR signalling pathway is therefore an important therapeutic target.

The human IgG1 monoclonal antibody VEGFR2 antagonist ramucirumab has been established as part of the standard of care in the second line treatment of metastatic gastric cancer. The REGARD study was a Phase III trial which compared ramucirumab with best supportive care in second line advanced gastric cancer, and showed an overall survival benefit of 1.4 months (5.2 vs 3.8 months, HR 0.776, 95% CI 0.603–0.998, p = 0.047) (Fuchs et al., 2014). The Phase III RAINBOW study compared the use of ramucirumab vs placebo, in combination with paclitaxel, in patients with advanced gastric cancer that had progressed on first line chemotherapy of fluoropyrimidine/platinum with or without an anthracycline (Wilke et al., 2014). There was a statistically significant overall survival benefit with ramucirumab of 9.6 months vs 7.4 months (HR 0.807, 95% CI 0.678–0.962, p = 0.017), with an increase of 1 year OS from 30% to 40%. Progression free survival, objective response rates (ORR) and disease control rates (DCR) were also improved. However, on subset analyses, the overall survival benefit of ramucirumab was not seen in Asian patients. The RAINFALL trial is in the process of looking at ramucirumab in combination with capecitabine and cisplatin in the first line therapy in patients with metastatic gastric cancer (NCT02314117). Bevacizumab, a VEGF directed monoclonal antibody, has also been studied, with less promising results. In the AVAGAST study, cisplatin and capecitabine were given with bevacizumab or placebo. There was no overall survival benefit from the addition of bevacizumab- median OS 12.1 months vs 10.1 months (HR 0.87, 95% CI 0.73–1.03, p = 0.1002), although PFS and ORR were improved (Ohtsu et al., 2011).

It is worth noting that the level of VEGF overexpression has not been conclusively shown to correlate with the benefits derived from VEGF pathway inhibitors. In the AVAGAST trial, patients with high plasma VEGF-A levels showed improved overall survival compared to those with low VEGF-A levels. Currently, ramucirumab is approved for use in second line gastric cancers, but the identification of a biomarker predicting response to anti-VEGF agents would allow us to better select appropriate patients for anti-angiogenic treatment.

3.3. EGFR

Overexpression of EGFR occurs in 2.3–40% of gastric tumours, and has been associated with poorer survival. Based on this, and given the success of both tyrosine kinase inhibitors and anti-EGFR monoclonal antibodies in other tumour types such as colorectal and lung cancer, studies have been done to investigate the benefits in gastric cancer (Dragovich and Campen, 2009).

So far, results have been disappointing. The EXPAND trial was a randomised phase III trial that evaluated the addition of cetuximab to the standard capecitabine and cisplatin in first line advanced gastric cancer. The study did not meet its primary endpoint of PFS improvement (HR 1.09, 95% CI 0.92–1.29, p = 0.32), with higher rates of toxicity. EGFR expression was generally low, with a median IHC score of zero, and there was no significant difference between treatment groups according to EGFR score (Lordick et al., 2013). In a similar patient group, the Phase III REAL3 study gave epirubicin, oxaliplatin and capecitabine with or without panitumumab, and in fact found a significant reduction in median overall survival with the addition of panitumumb (11.3 months vs 8.8 months, HR 1.37, 95% CI 1.07–1.76, p = 0.013). This study incorporated an exploratory biomarker analysis- in the 10 patients who had *KRAS* mutations, there was a non-significant trend towards benefit from the addition of panitumumab: OS HR 0.23 (95% CI 0.05–1.15) (Waddell et al., 2013). These studies highlight the fact that preselecting patients based on biomarkers may be the way forward in running trials of targeted agents.

A Phase III trial looking at nimotuzumab in combination with irinotecan as second line treatment is currently underway (NCT01813253). Likewise, while the small molecular tyrosine kinase inhibitor gefinitib showed some response in gastro-oesophageal junction tumours (overall response rate 9%), there was no response seen in the gastric cancers. No somatic EGFR mutations were found, and EGFR overexpression and Akt expression were not found to be predictive of response to gefinitib (Dragovich et al., 2006).

3.4. FGFR2

The fibroblast growth factor type 2 (*FGFR2*) gene located on chromosome 10q26 encodes the FGFR2 tyrosine kinase, which regulates cell proliferation and angiogenesis via activation of the MAPK, PI3K, STAT and phospholipase Cy signalling pathways. *FGFR2* amplification has been shown in various studies to occur in 3–16% of gastric cancers. It tends to be associated with diffuse type gastric cancers, later stage and poorer prognosis (Inokuchi et al., 2015).

Drugs targeting FGFR receptors have shown promise in preclinical studies as well as early clinical trials. One of the agents being actively studied is dovitinib (TKI258), a multikinase inhibitor against FGFR, VEGFR, PDGFR, FLT-3 (FMS-like tyrosine kinase 3), KIT and colony stimulating factor 1. Deng et al. tested the activity of dovitinib in *FGFR2*-amplified and non-amplified gastric cancer cell lines, and found that dovinitib decreased phosphorylation of FGFR2, ERK and AKT. There was potent growth inhibitory activity and reduction in colony formation in the amplified KATO III and SNU-16 cell lines but not in the non-amplified cell lines. Dovinitib also reduced mean tumour size in an *FGFR2*-amplified human gastric cancer xenograft model (Deng et al., 2015). There are currently a few clinical trials underway evaluating the use of dovitinib in advanced *FGFR2*-amplified gastric CA, including a Phase II trial of dovitinib monotherapy after failure of 2 lines of chemotherapy (NCT01719549), and a Phase I–II trial combining docetaxel with dovitinib after the failure of 1 line of chemotherapy (NCT01921673). The results of the SHINE trial, which looked at another FGFR2 inhibitor AZD4547 versus paclitaxel in advanced gastric cancer with *FGFR2* amplification, were recently reported. 9% of patients screened had *FGFR2* amplification. The overall median PFS on the AZD4547 arm was 1.8 months, compared to 3.5 months for paclitaxel, and in the 9% of patients with *FGFR* amplication, PFS was 1.5 months and 2.3 months respectively (NCT01457846) (Bang et al., 2015)

3.5. PI3K pathway

The PI3K-AKT-mTOR pathway is another frequently altered pathway in gastric cancer, either through activating mutations of *PIK3CA*, loss of the negative regulator *PTEN*, or amplification of *AKT*. Activation of this pathway can increase cell proliferation by promoting transcriptional activity, decreasing apoptosis and increasing cytoskeleton dynamics. The overall rate of *PIK3CA* mutations has differed greatly between studies, from just 0.8% to 20% overall in the TCGA study. This pathway can be targeted at the level of PI3K or downstream at AKT or mTOR.

Various PI3K inhibitors have been studied in gastric cancer, so far without definitive clinical results. Inhibitors like LY294002, BEZ 235 and BKM120 have been shown to have effect in preclinical studies (Xie et al., 2013; Mueller et al., 2012). Clinical trials are currently underway, often with PI3K inhibitors in combination with other drugs, including BYL719 together with HSP90 inhibitor AUY 922 in gastric cancer, and BKM120 with hedgehog pathway inhibitor LDE 225 in advanced solid tumours (NCT01613950, NCT01576666). The AKT inhibitor AZD5363 is being tested in the second line, in combination with paclitaxel in gastric cancer patients with and without *PIK3CA* mutations or amplifications (NCT02451956, NCT02449655). Another AKT inhibitor, GDC-0068, has been studied in the Phase II JAGUAR trial, in which patients with inoperable gastric or gastroesophageal junction adenocarcinomas are randomised to modified FOLFOX with or without GDC-0068. Results of this trial are awaited. (NCT01896531).

mTOR inhibitors have shown marked activity in other solid tumours, such as in renal cell carcinoma and breast cancer. In gastric cancer, a phase II study of everolimus had promising results, with a disease control rate of 54.7%, a median PFS of 2.7 months and a median OS of 10.1 months (Doi et al., 2010). This lead to the GRANITE-1 study, which evaluated everolimus vs placebo in patients with advanced gastric cancer that had progressed after one or two lines of systemic chemotherapy. However, everolimus did not show an overall survival benefit (5.4 months vs 4.3 months, HR 0.90, 95% CI 0.75–1.08, p = 0.124), although there was improvement in median PFS (1.7 months vs 1.4 months, HR 0.66, 95% CI 0.56–0.78) (Ohtsu et al., 2013). Biomarker analyses of GRANITE-1 are ongoing, as the identification of biomarkers may help define the patients who would benefit most from everolimus.

3.6. MET

MET amplification or overexpression has been found in 0–23% of gastric cancers on immunohistochemistry and in-situ hybridisation, representing a small but significant proportion of gastric cancers that may be amenable to targeting by MET inhibitors (Lee et al., 2012). AMG-337 is a c-MET inhibitor in the early phases of development that has shown promising results in gastric cancer- in the first-in-man Phase I trial, 8 patients with *MET* amplification were included (out of 45 in total)- 7 of these had gastro-oesophageal cancer, with 1 complete response that has lasted >3years, 4 partial responses and 1 stable disease (Hong et al., 2014). However, the phase II study of AMG337 has been halted since last year due to disappointing results. A phase II study is currently underway (NCT02016534). Two larger phase III trials in gastric cancer, for the c-MET inhibitors rilotumumab (an antibody against c-MET ligand hepatocyte growth factor) and onartuzumab (an antibody against the extracellular domain of c-MET) have been halted- in the case of rilotumumab, there was an increase in the risk of death from the study drug (Cunningham et al., 2013).

The disparate results between AMG-337 and rilotumumab/onartuzumab could be due to biomarker selection. In MetGastric, which evaluated the addition of onartuzumab to mFOLFOX6, and in RILOMET-1, which looked at the addition of rilotumumab to epirubicin, cisplatin and capecitabine, MET status was determined by IHC, and patients with 2+ or 3+ were included (NCT01662869). However, in the AMG337 studies, MET status was determined by fluorescence in situ hybridisation or next generation sequencing- this more accurate detection method may explain the stark responses seen. Other possibilities would include issues with the dose intensities of rilotumumab/onartuzumab- perhaps the combination with chemotherapy and its accompanying toxicities limits the optimisation of MET inhibitor dosage (Table 1).

4. Immunotherapy

Gastric tumorigenesis depends not only on mutations within the tumour cell itself, but also with its interaction with the surrounding immune environment (Hanahan and Weinberg, 2011). The ability of a tumour cell to escape immune detection and removal involves the programmed cell death protein-1/programmed death-ligand 1 (PD-1/PD-L1) pathway, as well as the interaction of CTLA4 with its ligands. When PD-L1 binds to its receptor on T lymphocytes, it inhibits T lymphocyte proliferation and induces apoptosis of tumour specific T cells, reducing the cytotoxic immune response towards the tumour. The TCGA study showed that EBV positive gastric cancers in particular over-expressed PD-L1 and PD-L2, which suggests that these tumours would be particularly susceptible to anti-PD1 therapy.

This was seen in the Keynote 012 trial, which was a Phase 1b study of anti-PD-1 IgG antibody pembrolizumab in patients with advanced solid tumours that stained positive for PD-L1 on immunohistochemistry, including a cohort of gastric cancer patients. 40% of patients screened were PD-L1 positive, and 67% had received >/ = 2

Table 1
Targets and therapeutic agents in gastric cancer.

Target	Therapeutic agents	Relevant studies	Line of treatment	Phase	OS
HER2	Trastuzumab	ToGA: fluoropyrimidine/cisplatin +/− trastuzumab	First	III	13.8 vs 11.1 months (HR 0.74, 95%CI 0.60–0.91)
	Lapatinib	TRIO-013/LOGIC: capecitabine/oxaliplatin +/− lapatinib	First	III	12.2 vs 10.5 months (HR 0.91, 95%CI 0.73–1.12)
		Tytan: Paclitaxel +/− lapatinib	Second	III	11.0 vs 8.9 months (HR 0.84, 95%CI 0.64–1.11)
	Pertuzumab	JACOB (NCT01774786): fluoropyrimidine/cisplatin/trastuzumab +/− pertuzumab	First	III	Study ongoing
	Trastuzumab-emtansine	GATSBY (NCT01641939): Trastuzumab emtansine vs taxan	Second	III	Study ongoing
VEGF	Ramucirumab	REGARD: ramucirumab vs best supportive care (BSC)	Second	III	5.2 vs 3.8 months (HR 0.776, 95%CI 0.603–0.998)
		RAINBOW: paclitaxel +/− ramucirumab	Second	III	9.6 vs 7.4 months (HR 0.807, 95%CI 0.678–0.962)
		RAINFALL (NCT02314117): capecitabine/cisplatin +/− ramucirumab	First	III	Study ongoing
	Bevacizumab	AVAGAST: capecitabine/cisplatin +/− bevacizumab	First	III	12.1 vs 10.1 months (HR 0.87, 95%CI 0.73–1.03)
EGFR	Cetuximab	EXPAND: capecitabine/cisplatin +/− cetuximab	First	III	9.4 vs 10.7 months (HR 1.00, 95%CI 0.87–1.17)
	Panitumumab	REAL3: epirubicin/oxaliplatin/capecitabine +/− panitumumab	First	III	8.8 vs 11.3 months (HR 1.37, 95%CI 1.07–1.76)
	Nimotuzumab	Irinotecan +/− nimotuzumab	Second	III	Study ongoing
FGFR2	Dovitinib	NCT01719549: dovitinib monotherapy	Third onwards	II	Study ongoing
		NCT01921673: docetaxel/dovitinib	Second onwards	I–II	Study ongoing
	AZD4547	NCT01457846: AZD4547 vs paclitaxel	Second	II	Study ongoing
PI3K	BYL719	NCT01613950: BYL719/AUY 922	Second to fourth	IB	Study ongoing
	BKM120	NCT01576666: BKM120/LDE 225	Beyond standard therapy	IB	Study ongoing
Akt	AZD5363	NCT02451956: AZD5363/paclitaxel	Second	II	Study ongoing
		NCT02449655: 2 arms-AZD5363/paclitaxel, AZD2014/paclitaxel	Second	II	Study ongoing
mTOR	Everolimus	GRANITE-1: everolimus vs BSC	Second onwards	III	5.4 vs 4.3 months (HR 0.90, 95%CI 0.75–1.08)
MET	AMG 337	NCT02016534: AMG337	Beyond standard therapy	II	Study ongoing
	Rilotumumab	RILOMET-1: epirubicin/capecitabine/cisplatin +/− rilotumumab	First	III	Study discontinued
	Onartuzumab	MetGastric: modified FOLFOX6 +/− onartuzumab	First	III	Study discontinued

prior therapies. Overall response rate was 22% by central review, and median response duration was 24 weeks, with a 6 month PFS rate of 24% and a 6 month OS rate of 69% (NCT01848834) (Muro et al., 2015). Nivolumab, another anti-PD1 antibody, is being tested in a Phase III trial in previously treated gastric cancer, which will not limit enrollment by PD-L1 biomarker status (NCT02267343). In addition, dual blockage of PD-1 and CTLA-4 is being evaluated, including in a phase Ib/II trial of nivolumab and nivolumab plus ipilimumab which is enrolling patients with metastatic gastric cancer (NCT01928394). MEDI4736, a human IgG1 monoclonal antibody against PD-L1, has also shown clinical activity in Phase I, as well as in early results from the expansion cohort, which includes patients with gastro-oesophageal cancers (Lutzky et al., 2014; Segal et al., 2014).

A recent study has shed light on the benefits of anti-PD-1 agent pembrolizumab in patients with mismatch repair deficient tumours, based on the hypothesis that these tumours have more somatic mutations and therefore may be more susceptible to immune checkpoint blockade. This study looked at pembrolizumab in heavily treated patients in three cohorts, and found that the benefits of pembrolizumab were most marked in patients with MMR deficient cancers. In the MMR deficient non-colorectal cohort, which included gastric cancer patients, immune PFS at 20 weeks was seen in 67% of patients, with a 71% immune overall response rate. The median PFS was 5.4 months and the median OS was not reached. This suggests that cancers with higher levels of somatic mutations have a higher response rate to PD-1/PD-L1 inhibition (Le et al., 2015). Of the various tumour types, gastric cancer has one of the highest levels of somatic mutations, trailing only melanoma, lung and bladder cancers (Lawrence et al., 2013). The role of immunotherapy in melanoma and lung cancers is already firmly established, and emerging information points to gastric cancer as the next frontier.

5. Conclusion

Our understanding of the genomics of gastric cancer has expanded significantly over the last few years. Sequencing advances have yielded many potentially targetable mutations, which in turn have led to the emergence of new therapies against these targets. Gastric cancer is a disease with a poor prognosis and limited treatment options, and these developments have given us new hope in improving patients' survival.

The most successful example of targeted therapy has been the use of trastuzumab, which has now been established as the standard of care in HER2 positive disease. In addition, exciting strides are being made in immunotherapy. Gastric cancer is striking in its high levels of somatic mutations, making it an ideal candidate for PD-1/PD-L1 blockade. It will be interesting to see how immunotherapy and other targeted therapies make advances beyond the later lines of treatment in advanced cancers, into the first line and even adjuvant settings.

Moving forward, there are still many challenges ahead. Firstly, although sequencing reveals druggable targets, not all of these have been successful in clinical testing. This is likely due to the fact that gastric cancers are genomically complex tumours, and not all mutations play the role of driver mutations. Targeted therapy may also be limited by the tumour heterogeneity seen in gastric cancers, and the existence of redundancy in the multiple pathways of carcinogenesis. Further work is needed to understand the central processes governing gastric cancer development, either to identify shared pathways for targeting, or if none, to better select personalised treatment.

Secondly, there is the need to identify appropriate biomarkers to select patients for treatment with targeted agents. Current

biomarker testing in trials tends to focus on one or two specific biomarkers, often using protein expression rather than genetic testing. Whole genome sequencing (WGS) is set to play an important role in this area, as it would allow us to widen the search for biomarkers in clinical trials of new therapeutic agents. In future, if more targeted agents emerge and biomarkers for their efficacy are recognised, sequencing to individualize treatment may become part of routine practice. WGS may also give us further insight into tumour heterogeneity. The focus now is on translating the diagnostic potential of this method into clinical practice, in particular in developing the expertise to deal with the large amounts of information yielded by WGS.

Finally, the development of molecular classification systems has called into discussion how and when to best apply them. One of the potential uses is in recruitment for trials- each subgroup is enriched with particular mutations and can be used to identify suitable patients. In the clinical setting, we need to establish stronger correlations between histological or clinical features with molecular features. If this can be done, classifications may be useful in prognostication and also in guiding our targeted molecular testing.

Conflict of interest

The authors do not report any conflicts of interest.

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